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Research Document 2001/097 | **Document de recherche 2001/097**

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High Levels of Genetic Variation in Northern Abalone *Haliotis kamtschatkana* of British Columbia

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This document is available on the Internet at: Ce document est disponible sur l'Internet à:
http://www.dfo-mpo.gc.ca/csas/

ISSN 1480-4883
Ottawa, 2001

Canada

1.0 ABSTRACT

Northern abalone (*Haliotis kamtschatkana*), from 18 sites in British Columbia and one site in southeastern Alaska, were surveyed for variation at 12 polymorphic microsatellite loci. In all samples, levels of observed heterozygosity were high (H_o = 0.64-0.74) but lower than values expected (H_e = 0.88-0.90) under conditions of Hardy Weinberg equilibrium (HWE), due to heterozygote deficiencies at all 12 loci. Levels of excess homozygosity varied more among loci (f_{is} = 0.02-0.55) than among samples (f_{is} = 0.16-0.28), indicating that inbreeding alone did not account for the large homozygote excess observed at some loci. Based on the six loci at which genotypic frequencies were closest to HWE expectations, the estimated level of inbreeding in northern abalone aggregations was 0.06. The high level of H_e characterizing all samples resulted in a large estimated effective population size for northern abalone (420,000), consistent with a high estimate for the historical average number of migrants entering abalone aggregations each generation (~20). Hierarchical analysis of gene diversity revealed that 99.6% of genetic variation was contained within abalone samples and only 0.4% partitioned among samples. Approximately 0.2% of variation was accounted for by differentiation between abalone of the Queen Charlotte Islands and Alaska and those found in central and southern British Columbia, and the remaining 0.2% was due to differences among samples within each of those two regions. The results indicate that, historically, northern abalone aggregations did not represent isolated breeding units and any disruption of gene flow that may have been caused by recent low abundance levels cannot yet be detected in non-size-structured samples of adult abalone.

1.1 RESUMÉ

On a étudié les variations de 12 loci de microsatellites polymorphes chez des ormeaux nordiques (*Haliotis kamtschatkana*) provenant de 18 endroits de la Colombie-Britannique et d'un site dans le sud-est de l'Alaska. Tous les échantillons présentaient des niveaux d'hétézygotie élevés (Ho = 0,64-0,74), mais inférieurs aux valeurs prévues en conditions d'équilibre Hardy-Weinberg (EHW), soit He = 0,88-0,90, en raison de déficits d'hétérozygotie aux 12 loci. Les niveaux d'homozygotie excédentaire variaient davantage entre les loci (coefficient de consanguinité = 0,02-0,55) qu'entre les échantillons (coefficient de consanguinité = 0,16-0,28), ce qui indique que la consanguinité ne peut expliquer à elle seule les grands excès d'homozygotie observés pour certains loci. En se fondant sur les six loci dont les fréquences génotypiques s'approchaient le plus des valeurs à l'EHW, on estime à 0,06 le niveau de consanguinité des concentrations d'ormeaux nordiques. En raison de la valeur élevée de He pour tous les échantillons, la taille effective estimée de la population était élevée (420 000), ce qui correspond à l'estimation élevée du nombre moyen des immigrants qui se joignaient par le passé à des concentrations d'ormeaux chaque génération (~20). L'analyse hiérarchique de la diversité génétique a montré que 99,6 % de la variation génétique se trouvait à l'intérieur des échantillons, contre seulement 0,4 % d'un échantillon à l'autre. Les différences entre les ormeaux des îles de la Reine-Charlotte et de l'Alaska, d'une part, et du centre et du sud de la Colombie-Britannique, d'autre part, représentaient environ 0,2 % de la variation inter-échantillons, tandis que le 0,2 % restant était attribuable aux différences entre les échantillons dans chacune de ces deux régions. Les résultats indiquent que les concentrations d'ormeaux nordiques ne constituaient pas des unités de reproduction isolées par le passé et qu'on ne peut pas encore détecter, dans des échantillons d'ormeaux adultes non classés par taille, de perturbation des flux géniques qui aurait été causée par les faibles abondances récentes de ce mollusque.

2.0 INTRODUCTION

Northern or pinto abalone (*Haliotis kamtschatkana*) are confined to the northeastern Pacific Ocean, inhabiting shallow (low intertidal to 30 m depth) coastal waters from southern California to Alaska. This abalone species occurs in patchy distribution on exposed and semi-exposed rocky coasts in British Columbia. The biology and ecology of *H. kamtschatkana* was reviewed by Sloan and Breen (1988). The abundance of northern abalone declined by 75-80% during 1978-90 and, for over a decade, remained low in spite of a complete harvest closure since 1990 in British Columbia (Campbell 2000). On 23 April, 1999, northern abalone was listed as a "threatened" species (i.e., one likely to become in imminent danger of extinction or extirpation if limiting factors are not reversed) by the Committee on the Status of Endangered Wildlife in Canada and has continued with this status to date (COSEWIC 2000). An examination of population structure in northern abalone has been undertaken as an initial step in the process of developing a comprehensive management plan for the species in British Columbia.

Species at low abundance partitioned into isolated small populations are at risk of extirpation and extinction from stochastic demographic, environmental and genetic factors. The biology of northern abalone indicates that this species may be especially vulnerable to processes in all three of these categories. The current low abundance and low densities of mature abalone (Campbell 2000) may reflect not only the commercial harvesting that occurred between 1970 and 1990, but also adverse environmental conditions that may have hindered successful recruitment over the period 1975-1983 (Breen 1986) and that may persist. In turn, the low abalone abundance hinders successful spawning because external fertilization requires high-density aggregations of mature individuals (Babcock and Keesing 1999). Finally, reduced spawning success may lead to the loss of genetic variation within local populations due to inbreeding and genetic drift, and the disruption of larval-mediated gene flow among local populations that might normally counteract the erosion of diversity within local populations.

The level and distance of larval dispersal are central to both demographic and genetic processes in sedentary marine organisms. Typically, levels of dispersal for abalone species are not known but are apparently sufficiently low to ensure that demographic processes occur on a local scale (i.e., recruitment is primarily local, ranging from a few m or km) and sufficiently high enough to prevent strong genetic differentiation over large geographic ranges (Brown 1991; Hamm and Burton 2000; Huang et al. 2000). Nevertheless, along the coasts of both southern Australia and California, genetic studies have provided evidence of different scales of population structure in sympatric abalone species, indicating that factors such as habitat utilization, spawning season, and larval duration may result in very different population structures among species. Alternately, Kyle and Boulding (2000) suggested that historical demographic events rather than differences in current levels of gene flow might underlie different degrees of population structure in two sympatric species of littorinid snails with planktonic larvae.

For blacklip abalone, *H. rubra*, sampled along the southern coastline of Australia, genetic data from allozyme, RAPD, minisatellite and microsatellite loci all indicated that there was 'isolation by distance', but that even the most geographically distant (>1000 km) populations were genetically similar. The F_{ST} value for this species estimated from allozymes was 0.022 (Brown 1991) and from microsatellites was 0.077 (Huang et al. 2000). Greater microspatial

genetic heterogeneity was observed in an Australian greenlip abalone, *H. laevigata*, a species with a more patchy distribution than *H. rubra*, at both allozyme and RAPD loci. However, whereas the allozyme study indicated some isolation-by-distance for *H. laevigata* the estimated F_{ST} value (0.014) was not greater than that of the blacklip abalone (Brown and Murray 1992; Shepherd and Brown 1993). Microspatial variability was also evident in the sympatric Roe's abalone, *H. roei*, a species demonstrating even greater genetic homogeneity over large distances. For this species, the F_{ST} value estimated from allozyme loci for samples collected over an almost 3000 km stretch of coastline was 0.009 (Hancock 2000).

Differences in population structure have also been observed in two sympatric abalone species along the coast of California. Samples of red abalone, *H. rufescens*, from northern and southern California were little differentiated at allozyme loci, in mitochondrial DNA sequence, or at a single microsatellite locus (Gaffney et al. 1996; Kirby et al. 1998; Burton and Tegner 2000). The F_{ST} value of 0.012 estimated among three samples from allozyme data was not significantly different from zero (Burton and Tegner 2000). In contrast, allozyme data for black abalone, *H. cracherodii*, indicated significant genetic differentiation among samples collected along the central Californian coast ($F_{ST} = 0.039$), a relatively high level of population subdivision that was attributed to a restricted spawning season that limits larval dispersal (Hamm and Burton 2000). These results led the authors to conclude that immigration from distant sources was unlikely to be sufficiently great to accelerate recovery in the depleted black abalone populations of southern California, estimated to have declined in abundance by as much as 97% (Altstatt et al. 1996).

The relatively low levels of intraspecific differentiation observed in abalone species has led to the suggestion that the partitioning of genetic diversity within abalone species may be most amenable to examination with highly polymorphic, rapidly evolving microsatellite loci (Huang et al. 2000; Withler 2000). In the present study, we survey variation at twelve polymorphic microsatellite loci in northern abalone collected from 18 sites in British Columbia and one site in southeast Alaska. We analyze the observed allelic and genotypic frequencies in the abalone samples to determine the levels of genetic variation within and among aggregations of abalone in British Columbia, and to estimate effective population sizes and inbreeding levels for the species. We examine allele frequency distributions for evidence of recent bottlenecks in population abundance that might have reduced genetic variation within, or increased variation among, extant abalone populations. We incorporate the genetic data into recommendations for conservation efforts likely to benefit the northern abalone of British Columbia.

3.0 MATERIALS AND METHODS

Epipodial tissue samples from adult abalone were collected from 18 sites within British Columbia and one site in southeast Alaska during 1998, 1999 and 2000 (Table 1, Fig. 1). SCUBA dive teams searched for emergent or exposed (visible on rocks) individuals because most are easily found, whereas immature abalone tend to be cryptic (Campbell 1996). Samples from abalone within 10-200m were used to represent each collection area. The small epidodial tissue sample removal from each abalone was considered non-destructive, causing no mortality to the abalone (A. Campbell unpublished data on a laboratory experiment). In addition to the 95

tissue samples collected from Denman/Chrome Island in 2000, tissue samples were taken from 45 illegally harvested abalone putatively collected near Chrome Island in the Strait of Georgia in 1999 (Fig. 1). Samples were stored in 95% ethanol prior to DNA extraction using DNeasy kits (Qiagen, Valencia, CA).

Variation at 12 microsatellite loci isolated from northern abalone was surveyed using the primers and protocols outlined by Miller et al. (in press). For each abalone sampled, alleles were amplified for each locus using the polymerase chain reaction (PCR) and sized using standard electrophoretic techniques on an ABI 377 automated DNA sequencer. The 12 microsatellite loci consisted of simple and compound di-, tri- and tetra-nucleotide repeat sequences. Alleles at each locus were generally differentiated by the number of basepairs (bp) of the predominant repeat unit, but alleles differentiated by a single bp were observed at some of the complex loci (Table 2).

Analysis of the allelic and genotypic frequency data was carried out using the Genetic Data Analysis (GDA) program of Lewis and Zaykin (2000) and GENEPOP version 3.1d (Raymond and Rousset 1995). Genotypic frequencies at each locus in each sample were tested for conformance to Hardy Weinberg equilibrium (HWE) distributions in GENEPOP. F_{ST} and Nei's (1972) genetic distance values were computed using GDA among all samples. The significance of multilocus F_{ST} values was determined by bootstrapping over loci and of single-locus F_{ST} values by jackknifing over samples. In GDA, F_{ST} was calculated for multiple alleles and loci according to Weir and Cockerham (1984). Non-zero estimates of F_{ST} values for a group of samples indicate that the individuals of each sample are more closely related to each other (i.e. have a more recent common ancestor) than they are to individuals of the other samples. Nei's (1972) genetic distance is a standard distance measurement based on differences in allele frequencies between samples. Cavalli-Sforza and Edward's (1967) chord distance among samples was computed using PHYLIP (Felsenstein 1993). GENEPOP was used to perform Mantel's (1967) regression of the pairwise linearized F_{ST} values $[(1-F_{ST})/F_{ST}]$ on the natural logarithm of geographic distance to test for 'isolation by distance' among abalone samples.

The genetic distance calculated from the six loci with the smallest heterozygote deficiencies relative to HWE expectations and the chord distance values calculated from all twelve loci were independently clustered with the neighbor-joining algorithm to provide dendrograms of the genetic relationships among abalone samples. The pairwise average number of migrants (Nm) between samples was estimated by the private alleles method of Barton and Slatkin (1986) using GENEPOP and with the expression $F_{ST} = 1/(4Nm + 1)$, a relationship based on the assumption of island model of population structure (Whitlock and McCauley 1999). The effective population size (N_e) for northern abalone was calculated from expected heterozygosity (H_e) values for the 12 microsatellite loci using the relationship $N_e = (1/[1-H_e]^2-1)/8\mu$, where μ is the mutation rate for the microsatellite loci (Lehmann et al. 1998). Little is known of the mutation rate of microsatellite loci in invertebrate organisms except *Drosophila*, in which the observed rate ($\sim 10^{-6}$) is much lower than in mammals ($\sim 10^{-4}$). N_e for northern abalone was estimated in this study using the conservative assumption that $\mu = 10^{-4}$, with recognition that N_e values are 100 times greater if the true value is 10^{-6}.

Hierarchical analyses of allele frequency variation were carried out with nested ANOVA (random effects model) as described by Weir (1996). The proportions of the observed variation attributable to regions (the Queen Charlotte Islands [QCI], central coast of British Columbia, west coast of Vancouver Island and Georgia Strait), to sample sites within regions, and to genetic variation within sample sites were determined. Geographic variation was examined with two models. The four-region model examined variation among the four regions listed above (samples shown by region in Table 1). The two-region model examined differentiation between the QCI and the Alaskan sample, which were distinctive in the dendrograms, and all other samples. Both models were used with the data from all 12 microsatellite loci and with the data from six loci at which genotypes mostly closely approximated HWE frequencies.

Heterogeneity among cohorts (age classes) within samples was investigated in the samples from five sites. Abalone were divided into four size classes based on shell length (SL): ≤50 mm (immature), 51-69 mm (transition of immature to mature), 70-99 mm (mature) and >99 mm (fishery), defined by size at maturity estimates by Campbell et al. (1992). Each of these size groups contained a range of ages whose growth rates could have been influenced by local environmental conditions: ≤ 2 to ≤ 4 years (≤50 mm SL), between 2 and 7 years (51-69 mm SL), between 3 and 14 years (70-99 mm SL) and >6 or >14 years (>99 mm SL) estimated from Sloan and Breen (1988 see Fig. 8). The maximum age of *H. kamtschatkana* is not known, but individuals reach ages of 30 years and older (Breen 1980). Thus, the potential number of cohorts contained within each size class increases with size class. Allele frequencies in the two or three size classes containing the most abalone at each site were analyzed by ANOVA to examine the possibility that small numbers of adults contribute to recruitment in individual cohorts of northern abalone, leading to low genetic variability within cohorts and significant variation among cohorts within abalone aggregations.

4.0 RESULTS

4.1 *Genetic variation within populations*

All microsatellite loci examined were highly polymorphic, exhibiting high numbers of alleles and high values of both observed (H_o) and expected (H_e) (under conditions of HWE) heterozygosities (Table 2). Genotypes at all twelve loci showed a significant excess of homozygotes in comparison to those expected under HWE, but the level of heterozygote deficiency varied greatly among loci (Table 2). Estimates of f_{is} (the level of inbreeding if the excess of homozygotes was due entirely to assortative mating among relatives) ranged from 0.02 at *Hka*43 to 0.55 at *Hka*85.

All 20 samples of northern abalone displayed high levels of genetic variability. Allelic diversity (mean numbers of alleles observed over all loci) was high and relatively constant among samples (Table 3). Average H_o by sample ranged from 0.64 – 0.74 (mean of 0.71), but in all cases was less than the H_e which was essentially 0.89 for all samples (Table 3). Thus, the estimated f_{is} value varied much less among samples (from 0.16 to 0.28) than among loci. The great range of f_{is} values among loci and the consistency of the f_{is} values for a given locus among samples indicate that inbreeding is not the sole explanation for the observed heterozygote deficits.

Using the mammalian microsatellite mutation rate (10^{-4}) and H_e values estimated for the abalone microsatellite loci of this study, we obtained locus-specific estimates of effective population size (N_e) ranging from 10,000 to 1,390,000, and a mean value of 420,000 (Table 2). Use of the possibly more realistic mutation rate of 10^{-6} provides estimates 100 times larger.

4.2 *Genetic variation among samples*

The F_{ST} value calculated from all 12 loci among all samples was low but significantly greater than zero (0.003; SE 0.0007). Calculated from the six loci closest to HWE that could be consistently scored in over 80% of abalone among samples (*Hka*12, *Hka*37, *Hka*40, *Hka*43, *Hka*56 and *Hka*65), the F_{ST} value was reduced but remained significantly greater than zero (0.002; SE 0.0006). Examined on a single locus basis, F_{ST} values ranged from 0.0 to 0.010, and were significantly greater than 0 for 8 of the 12 loci examined (Table 2).

There was not a strong geographically-based grouping of samples apparent in either the dendrogram based on CSE chord distance calculated from 12 loci (Fig. 2) or the dendrogram based on Nei's genetic distance values calculated from 6 loci (Fig. 3). In both dendrograms, the six QCI and single Alaskan sample clustered together, but the central coast, Georgia Strait and west coast Vancouver Island samples did not cluster geographically. In both dendrograms the two samples from the west coast of Vancouver Island (Elbow Island and Vargas Island) not only failed to cluster together, but one (Elbow Island) clustered with the QCI samples whereas the other (Vargas Island) clustered with the non-QCI samples. In contrast, the two samples from Chrome/Denman Island (1999 and 2000) did cluster together, albeit not strongly (as indicated by the relatively long branch distances joining them). However, because the 1999 sample consisted of illegally harvested abalone and was attributed to the Chrome Island site by hearsay, the significance of this relationship cannot be evaluated. The central coast samples did not form a strong group based on either the 6-locus or the 12-locus analysis. None of the nodes in the CSE dendrogram was supported by a bootstrap value of greater than 50%, indicating weak support for the depicted sample structure.

The hierarchical analyses of gene diversity indicated that 99.6% of the observed genetic variation occurred within samples and only 0.4% was attributable to differentiation among samples (Table 4). Of the differentiation among samples, approximately half (0.2%) was due to differences among regions and the other half to differences among samples within regions. This result did not vary among the various models used to test regional and sample differentiation (Table 4). Thus, the use of the six loci listed above at which genotypes within samples were closest to HWE expectations gave the same result as the use of all 12 loci. Similarly, including the single Alaskan sample from Sitka Sound in model as a member of the QCI region did not change the proportion of variation accounted for by region. Finally, region accounted for 0.2% of the total variation in both the four-region and two-region models, but the effect of region was not significant in either the four-region ($F_{3,16} = 1.75$, $P > 0.10$) or the two-region model ($F_{1,18} = 2.53$, $P > 0.10$). Thus although it was primarily the differentiation between QCI and non-QCI samples that accounted for the regional variance component, the distinction in allele frequencies between the two regions was small. Similarly, regardless of the model or number of loci used, the variation among samples within region was not significant (all $P > 0.05$).

The correlation of the linearized F_{ST} values with geographic distance approached significance (P=0.06), but geographic distance accounted for very little of the observed variation in F_{ST} values ($r^2 = 0.05$)(Fig. 4). Again, pairwise F_{ST} values tended to be less in comparisons of QCI samples with each other (0.003), non-QCI samples with each other (0.003), than between QCI and non-QCI samples (0.004). The distinctiveness of the QCI and Alaskan samples and their relatively great geographic distance from many of the remaining samples accounts for the weak relationship between geographic and genetic differentiation.

The average number of migrants per generation into the abalone aggregations represented by each sample was estimated by the private alleles method as 19.9, a number consistent with the observed lack of genetic differentiation among samples. This value changed little when only QCI/Alaskan samples (18.4) or only non-QCI samples (20.3) were considered. Calculating the average number of migrants using the standard expectation for the relationship between F_{ST} and Nm provided an estimated 83 migrants entering abalone aggregations each generation.

4.3 *Genetic variation between size (age) groups within samples*

F_{ST} values calculated over two or three size (age) samples of abalone from five sites averaged 0.001, and ranged from 0 to 0.027. Allele frequencies did not differ significantly in pairwise comparisons between size classes within each site. The numbers of alleles observed in the smaller (younger) abalone at each site tended to be less than in the older abalone (Table 5). This was likely due to the smaller sample sizes of younger abalone rather than a real reduction in allelic diversity because the expected heterozygosity values were the same among size classes (Table 5). The data provided an indication that levels of inbreeding within the smaller size classes, composed of fewer cohorts, was greater than in larger size classes. Only in the sample from Hankin Point, was the inbreeding level higher in the larger abalone (Table 5). Thus, the inbreeding observed in northern abalone likely results from the settlement and recruitment of abalone in locations very close to their parents. However, the fact that the smaller size classes retained high levels of allelic diversity and that age groups were not strongly differentiated in allele frequencies indicated that the number of abalone participating in individual spawning events was not extremely low.

5.0 DISCUSSION

This study provides the first analysis of genetic structure in *H. kamtschatkana* and the first comprehensive examination of population structure in any abalone species based on microsatellite variation. The study provided evidence for very high levels of genetic variation within northern abalone aggregations and very low levels of differentiation among samples collected from throughout British Columbia, including a single sample from southeast Alaska. Fully 99.6% of the observed variation was contained within samples, with only 0.2% attributable to two regional groupings of abalone (QCI/Alaska vs non-QCI) and 0.2% attributable to variation among the samples within those two regions. The lack of strong differentiation among samples and weak evidence for 'isolation by distance' suggests that gene flow among abalone breeding aggregations throughout British Columbia is, or has been, extensive. If recent low levels of abundance have disrupted historical patterns of gene flow, it is not yet evident among mature abalone of the age groups encompassed in this study.

The abalone of the QCI were slightly differentiated from abalone in other areas of British Columbia, perhaps as the result of restricted larval exchange in oceanographic currents, or perhaps because of historical isolation. During the last Cordilleran glaciation of North America, which ended approximately 12,000 years ago, the QCI and Alaskan coastal regions may have provided refugial habitat for terrestrial and marine organisms (Warner et al. 1982). Northern abalone throughout much of coastal British Columbia and those of the QCI (and perhaps northern BC and southeast Alaska) may be descendants of different refugial populations. Two distinctive clades in mitochondrial DNA sequences of the littorinid snail *Littorina subrotundata* throughout British Columbia and Washington have been attributed to dispersal from separate glacial refugia (Kyle and Boulding 1998, 2000). An examination of mtDNA variability in northern abalone might enable determination if the small degree of differentiation at microsatellite loci is due to historical isolation in separate refugia or more recent restrictions of gene flow between coastal and QCI habitats. Even if extant abalone are descendants of different refugial populations, the high level of intraspecific variability and low level of intersample differentiation indicate that refugial population sizes were large and limited genetic divergence occurred during isolation, or that gene flow has occurred since the glacial period.

All of the microsatellite loci examined in this study exhibited an excess of homozygosity relative to genotypic distributions expected under Hardy Weinberg equilibrium conditions. Homozygote excess has been observed in other population surveys of abalone at microsatellite and allozyme loci and was generally attributed to inbreeding (Huang et al. 2000; Brown 1991; Hara and Kikuchi 1992). In this study, variation among loci in the level of heterozygote deficiency observed makes it likely that locus-specific factors are also involved. As in the study of Huang et al. (2000), we tested multiple sets of primers for each locus in an attempt to eliminate the problem of non-amplifying alleles that result from mutations in flanking sequences used for primer design. For some of the loci in this study, judicious choice of primers reduced or eliminated non-amplifying alleles, whereas for other loci, the various primer sets tested all gave equivalent levels of excess homozygosity. Levels of excess homozygosity differed little among samples, with the f_{is} values (ranging from 0.16-0.28) merely reflecting the average of the f_{is} values among loci (0.21). Thus, some level of inbreeding may occur in northern abalone, as in other abalone species, the level of which is best estimated by those loci showing the least evidence of non-amplifying alleles (i.e. those loci with genotypic frequencies closest to HWE). At two loci, *Hka*3 and *Hka*85, only approximately half of the individuals surveyed were heterozygous although the high levels of polymorphism indicated that virtually all individuals should be heterozygous under HWE conditions. One or both of these loci may be located on only one member of a dimorphic pair of sex chromosomes, so that the gender which carries heteromorphic sex chromosomes is hemizygous for the locus (i.e. carries an allele on only one sex chromosome) but was scored as homozygous in the present study. Although both *Hka*3 and *Hka*85 may be located on a sex chromosomes they were in linkage equilibrium (i.e. they are not linked to each other)

Six of 12 loci could be consistently scored in all samples and exhibited f_{is} values of 0.10 or less, with an average value of 0.06. This may represent the typical level of inbreeding in northern abalone populations. High levels of local larval recruitment and/or asynchronous spawning on a small geographic scale may contribute to inbreeding in *H. kamtschatkana*, as

suggested for blacklip abalone (Huang et al. 2000). The analysis of inbreeding among size classes in this study indicated that inbreeding tended to be less in larger size classes (potentially containing individuals from more cohorts) than in smaller size classes (potentially containing individuals from fewer cohorts). Thus, relatedness may be greater among the recruits within a site from individual spawning events than among those from different years. Recruitment may be very local with progeny settling near their parents and/or the number of individuals that spawn successfully at any one time may be small. To some extent, these results are consistent with a model of "sweepstakes-style" recruitment success (Hedgecock 1994). According to this model, only a small fraction of mature adults effectively contribute to reproduction in each generation because of a limited window of oceanographic conditions compatible with successful spawning and/or recruitment. The resultant spatial and temporal variability in recruitment success may lead to detectable genetic drift among cohorts and to 'chaotic genetic patchiness', in which samples in very close proximity are as genetically differentiated as ones very far apart (Larson and Julian 1999). Although proximal samples of northern abalone in this study were sometimes as different as distal ones, all samples were highly polymorphic and little differentiated. Moreover, F_{ST} values were on average less (0.001) among age classes within a site than among sites (0.002), and in no case was the F_{ST} value among age classes within a site significantly greater than zero. Thus, there was little evidence that the successful spawners at any given time were sufficiently small in number or closely related to result in genetic drift.

Moberg and Burton (2000) found significant spatial and temporal genetic variability among and within three size classes of red sea urchin (*Strongylocentrotus franciscanus*) along the coast of California. Adult, sub-adult and recruit urchins collected from the same site frequently possessed significantly different allozyme frequencies, a finding largely attributed to inter-family variation in reproductive success. For northern abalone, the pooling of multiple ages (recruitment events) in each of the size classes examined may have obscured variation in individual spawning seasons, but the low levels of differentiation observed among sites over the 1200 km range examined in this study supports the indication that stochastic variation in reproduction/recruitment may be less in northern abalone than in sea urchin species.

All samples of northern abalone in this study were characterized by a high abundance of rare alleles (>80% of alleles were present at frequencies < 0.1), thus producing L-shaped distributions of allele frequencies. This indicates that populations have existed at long-term stable sizes (i.e., not suffered recent bottlenecks) (Luikart et al. 1998). The high values of heterozygosity (H_e) observed for northern abalone in this study led to high estimates of effective population size, indicating that the small local aggregations of mature abalone observed in census studies (Campbell 2000; Wallace 1999) did not represent genetically isolated breeding units. 'Cryptic' abalone, not recently included in census counts, possibly also contribute to reproduction in northern abalone. However, it is evident that local northern abalone aggregations are, or have been, connected by gene flow as the result of larval dispersal. Spawning is seasonal, usually restricted to summer months (i.e. May-August), and the pelagic larval stage is of short duration, although it varies (4 to 8 days) with local factors such as temperature (14 to 10°C) (Sloan and Breen 1988).

The finding that larval dispersal has been sufficient to prevent strong genetic differentiation throughout much of coastal British Columbia in an abalone species in which

spawning is seasonal is contrary to the results for another seasonal spawner, the black abalone, in California (Hamm and Burton 2000). In that species, F_{ST} values ten times higher than those estimated in this study were obtained from variation at three allozyme loci and the level of population differentiation was attributed at least in part to the limited spawning season and strong seasonal differences in oceanographic patterns in the coastal waters of California. The lack of genetic structure in northern abalone is more similar to the low level of genetic differentiation observed in the red abalone of California, which spawns throughout the year (Burton and Tegner 2000) and in three sympatric abalone species inhabiting the waters of southern Australia. The Australian blacklip, greenlip and Roe's abalone all show low levels of genetic differentiation over spatial scales as large or larger than those encompassed in the present study (Brown 1991, Brown and Murray 1992, Hancock 2000). For *H. roei*, the F_{ST} value estimated from eight allozyme loci among samples collected over almost 3000 km was 0.009. As for northern abalone, F_{ST} values between proximate samples of Roe's abalone could be as great as between distal samples, especially over distances of 1200 km or less. However, the greater differentiation of samples separated by more than 1500 km in that species gave a clear indication of isolation by distance over large distances. Hancock (2000) suggested that the small-scale heterogeneity in allele frequencies in Roe's abalone was due to predominantly local recruitment, with the high gene flow resulting more from large effective population sizes than from large migration rates. Moreover, he suggested that rare cases of successful long-distance dispersal might play a role in maintaining the observed large-scale genetic homogeneity.

For northern abalone, in which the mean F_{ST} value (0.002) was even lower than in Roe's abalone, large effective population sizes likely have also contributed to the observed genetic homogeneity. However, estimates of the average number of successful migrants among the samples of this study were also relatively large. Whether successful larval dispersal in the northern abalone occurs on a regular basis or is predominantly the result of rare, but highly effective, long distance dispersal events is not known. In either case, the lack of genetic differentiation at neutral genetic markers such as microsatellite loci does not preclude the possibility of adaptive genetic differentiation over the range. Although small breeding aggregates of northern abalone clearly do not represent strongly isolated subpopulations, adaptation to environmental conditions may be assumed to occur on a broad regional basis.

Long-lived species may maintain genetic variation even in the face of fluctuating environments and recruitment because of the "storage capacity' that results from the large cohort of adults produced from each strong recruitment (Warner and Chesson 1985; Ellner and Hairston 1994; Ellner 1996; Gaggiotti and Vetter 1999). These species effectively 'store' a large number of genotypes within the reproductive population over many reproductive periods that are capable of contributing to both population size and genetic diversity when favourable spawning and recruitment conditions return. However, extended periods of low reproductive/recruitment success may be masked in genetic surveys heavily influenced by the genetic variability being stored in, but not transmitted from, the older age groups. Gaggiotti and Vetter (1999) suggest that even when marine fisheries collapse, exploited species may be close to extinction as the result of demographic or environmental stochasticity before a marked reduction in genetic variation occurs. For a species such as northern abalone, in which historical effective population sizes have been large and the reservoir of neutral genetic variation in the older size classes is correspondingly great, genetic surveys conducted on a non-size-structured basis may fail to

detect population fragmentation and increased genetic drift due to low abundance levels if and when they occur. Early detection of genetic changes would be facilitated by size-structured analysis of samples on the finest scale achievable.

The analysis of genetic variation in different size (age) classes of abalone at several sites in this study provided no indication that younger age classes were less diverse than older ones, but the sampling of the younger ages was restricted and did not include newly recruited 'cryptic' individuals. In the black abalone of southern California, recruitment failure was observed after abalone abundance dropped by approximately 50% (Richards and Davis 1993). Because of the 'storage capacity' in the older individuals of abalone populations, it is essential that recruitment be measured to determine current levels of reproductive success. Longterm genetic monitoring of newly recruited abalone would reveal the loss of genetic diversity and population fragmentation that might follow a disruption of gene flow at low abundances, but only some years after the fact.

As for other overfished marine organisms, efforts to rebuild depleted abalone populations by out-planting of hatchery-reared larvae or juveniles have been largely unsuccessful. In addition to considerable technical problems with producing healthy seed, out-planted abalone tend to experience high mortality due to natural or human predation (Tegner 2000; Shepherd et al. 2000). Nevertheless, given good quality seed, favourable environmental conditions and sufficient protection after out-planting, there may be circumstances under which enhancement can contribute to stock rebuilding efforts (Shepherd et al. 2000). Genetic concerns associated with enhancement include the possibility of disrupting natural populations by out-planting abalone from a distant source that are adapted to different environmental conditions. Such abalone may not survive to reproduce but, if they do, threaten the genetic integrity of natural abalone populations in the transplant area (Tringali and Bert 1998, Shaklee and Bentzen 1998; Utter 1998). For northern abalone, transplantation should be confined within major coastal regions (e.g. Strait of Georgia, west coast of Vancouver Island, central coast, Queen Charlotte Islands) in order to avoid the introgression of maladaptive genes into natural populations. Two other concerns associated with the out-plant of hatchery produced organisms are the random loss of genetic diversity due to a limited number of spawners and, if the broodstock is maintained in the hatchery over generations, the development of a strain that is not well adapted to survival and reproduction in the wild. Hatchery strains that are intended for reseeding into natural populations should be carefully monitored to ensure that high levels of genetic variation are maintained, and should be open populations that incorporate naturally produced individuals on a regular basis. Genetic monitoring may also contribute to evaluation of the success of enhancement efforts (Burton and Tegner 2000).

The northern abalone of British Columbia likely constitute a single evolutionarily significant unit (ESU) (Moritz 1994), although additional genetic investigation may establish the abalone of the QCI and northern British Columbia as an independent ESU. The microsatellite data of this study indicate that an extremely high level of genetic variation exists in adult aggregates throughout the province. This reflects historical and/or current gene flow that has been of sufficient magnitude to prevent population subdivision even in the face of highly localized larval recruitment and resultant inbreeding levels of ≥ 0.06. Nevertheless, stock assessment data for areas in close proximity experiencing different environmental conditions and

levels of harvest restriction indicate that the demographic characteristics of abalone aggregates can be independent on small geographic scales (for literature reviews see Sloan and Breen 1988; Wallace 1999; Campbell 2000). The migration rate estimated from the microsatellite data of this study is not sufficiently high to preclude the possibility of local adaptation (genetic differentiation at loci under natural selection) among abalone inhabiting different environments within British Columbia. The current picture of northern abalone, as a collection of spawning aggregates connected by gene flow, indicates that population abundance levels have not been low for sufficiently long time periods to result in the loss of genetic diversity within aggregates or to cause random genetic differentiation among aggregates. Nevertheless, whereas the data indicate that extant spawning aggregates large enough to have been sampled in this study contain high levels of genetic variation, they can not be used to determine if current levels of reproductive success and gene flow are sufficient to maintain the diversity. Prudent management activities would include the identification, protection and monitoring of spawning aggregates (and recruits) on a regional basis to examine both demographic and genetic parameters for signs of population recovery or decline.

6.0 RECOMMENDATIONS

1. This study has shown that northern abalone aggregates are not genetically depauperate or heavily inbred, and have the genetic capacity for population expansion when favourable environmental conditions prevail. Since adult northern abalone are sedentary, and the stock recruitment relationships and larval dispersal mechanisms are unknown and difficult to assess, rehabilitation management should focus on preserving existing brood stock *in situ*, perhaps establishing a number of reserve areas where increased protection of local populations is possible.

2. Attempts to re-establish or enhance abalone aggregates through out-planting have generally been found to be unsuccessful when evaluated rigorously. The most promising method to increase local population abundance may be to ensure successful fertilization/reproduction in as many areas as possible. Techniques such as artificially aggregating adult abalone in areas of low density or the out-planting of hatchery-reared juveniles may be useful but would require careful monitoring for evaluation.

3. The number of abalone broodstock used to produce larvae or juveniles for out-planting to the wild should be at least 50 and preferably 100, with equal numbers of males and females, in order to maintain genetic diversity in the enhanced population. Controlled single pair mating and/or genetic monitoring of the progeny produced in the hatchery should be undertaken to maximize the number of parents contributing to juvenile production.

4. Out-planting and/or transplanting of northern abalone within British Columbia should be carried out within existing management regions (west coast Vancouver Island, east coast Vancouver Island, central coast and the Queen Charlotte Islands) to avoid the introgression of deleterious genes into natural populations, as well as reduce the possibility of disease transfer.

5. Research to determine the effect of low abundance on genetic structure in northern abalone and evaluation of enhancement efforts will both require the ability to measure and sample recruitment into abalone aggregates. Establishing study sites in which larval dispersal, recruitment and microspatial and temporal genetic variability could be monitored would enable greater understanding of the current population genetics and demographics of northern abalone.

7.0 ACKNOWLEDGEMENTS

We thank S. Carignan, B. DeFrietas, J. Disbrow, R. Gurr, J. Harding, M. McNab, T. Norgard, D. Miller, and D. Woodby for help with sample collections, and Dr. R.S. Burton for a helpful review of this paper.

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Table 1. Locations and years in which adult *H. kamtschatkana* were sampled for microsatellite analysis.

Site	Latitude	Longitude	Year
West coast Vancouver Island			
Elbow Island	48 54.060	125 16.556	2000
Vargas Island	49 09.429	125 57.729	2000
Georgia Strait			
Chrome/Denman Island	49 28.883	124 41.209	1999, 2000
BC central coast			
Cranstown Point	51 22.500	127 46.500	1999
Nalau Passage	51 47.000	128 06.500	1999
Simonds Group	51 57.800	128 16.700	1999
Iroquois Island	52 02.895	128 19.445	1999
Stryker Island	52 05.990	123 23.207	1999
Nowish Islands	52 31.000	128 26.000	1999
Higgins Pass	52 28.500	128 45.500	1999
Lotbiniere Bay	53 00.000	129 33.000	2000
Hankin Point	54 42.400	130 24.000	2000
Queen Charlotte Islands			
Louscoone Inlet	52 07.692	131 14.127	1999
Montserrat Bay	52 06.227	130 59.170	1998
Skincuttle inlet	52 20.780	131 14.260	1998
Faraday Island	52 36.770	131 27.800	1998
Virago Sound	54 04.000	132 31.000	1998
Bruin Bay	54 10.017	132 58.752	1999
Alaska			
Sitka Sound	57 03.100	135 20.500	1999

Table 2. Microsatellite loci surveyed in *H. kamtschatkana* from 19 sites in British Columbia and Alaska. The nature of the microsatellite repeat is shown as di (dinucleotide), tri (trinucleotide), tetra (tetranucleotide) or compound. The number of alleles (A) and size range (in basepairs) for each locus are also shown. The mean levels of observed (H_o) and expected (H_e) heterozygosity, estimated levels of inbreeding (f_{is}) and variation among samples (F_{ST}) are shown for each locus. The effective population size (N_e) of northern abalone estimated from the H_e values is shown.

Locus	Repeat	A	Size Range	H_e	H_o	F_{ST}	f_{is}	N_e/1000
*Hka*3	Compound tri-tetra	74	200-350	0.97	0.45	0.003 *	0.53	1390
*Hka*6	Compound tri-tetra	32	107-206	0.75	0.47	0.010 *	0.38	20
*Hka*12	di	81	171-377	0.93	0.89	0.0002	0.04	250
*Hka*28	di	38	183-271	0.95	0.57	0.002 *	0.40	500
*Hka*37	Compound tri-tetra	29	236-330	0.70	0.66	0.004 *	0.06	10
*Hka*40	di	36	112-210	0.92	0.86	0.002 *	0.07	190
Hka43	tetra	24	163-263	0.88	0.87	0.006 *	0.02	90
*Hka*48	di	73	93-250	0.97	0.50	0.003 *	0.22	1390
*Hka*56	di	32	93-160	0.92	0.84	0.0	0.08	190
*Hka*65	di	59	115-250	0.95	0.85	0.001	0.10	500
*Hka*80	di	25	89-150	0.93	0.88	0.005 *	0.04	250
*Hka*85	tri	45	200-390	0.92	0.42	0.002	0.55	200
Mean	-	45.7	-	0.90	0.71	0.003 *	0.21	420

* Significantly greater than 0 at α=0.05

Table 3. Genetic variation at 12 microsatellite loci in 20 samples of *H. kamtschatkana* from British Columbia and Alaska. The sample size (N), mean number of alleles observed per locus (A), observed (H_o) and expected (H_e) levels of heterozygosity, and estimated level of within sample inbreeding (f_{is}) are shown.

Site	N	A	H_e	H_o	F_{is}
WC Vancouver Isl					
Elbow Island	45	20.0	0.88	0.64	0.28
Vargas Island	70	24.4	0.89	0.72	0.20
Georgia Strait					
Chrome Island	70	22.9	0.88	0.74	0.16
Chrome Island*	45	20.7	0.89	0.70	0.21
BC central coast					
Cranstown Point	110	27.9	0.90	0.73	0.19
Nalau Passage	115	28.8	0.89	0.70	0.21
Simonds Group	125	26.3	0.89	0.70	0.21
Iroquois Island	110	29.3	0.90	0.71	0.21
Stryker Island	90	24.8	0.88	0.70	0.21
Nowish Islands	112	27.7	0.89	0.71	0.20
Higgins Pass	90	25.4	0.88	0.71	0.20
Lotbiniere Bay	28	16.0	0.88	0.65	0.27
Hankin Point	130	28.7	0.89	0.73	0.18
Queen Charlotte Isl					
Louscoone Inlet	130	30.4	0.89	0.69	0.22
Montserrat Bay	70	25.7	0.89	0.71	0.20
Skincuttle Inlet	73	25.8	0.89	0.70	0.21
Faraday Island	72	25.2	0.90	0.70	0.21
Virago Sound	70	24.4	0.90	0.73	0.18
Bruin Bay	90	28.5	0.90	0.73	0.19
Alaska					
Sitka Sound	95	27.8	0.90	0.70	0.23
Total/Mean	1740	25.5	0.89	0.71	0.21

* sample of confiscated abalone attributed to Chrome Island site

Table 4. Hierarchial analyses of genetic diversity for 20 samples of *H. kamtschatkana* from British Columbia and Alaska. Regions consisted of the Queen Charlotte Islands [QCI], central coast of British Columbia, west coast of Vancouver Island and Georgia Strait in the four-region model, with the sample from southeast Alaska included in the QCI region or excluded from the analysis (BC only). Regions consisted of the QCI and the remainder of British Columbia in the two-region model, with the Alaskan sample included in the QCI region or excluded from the analysis (BC only).

Model	Absolute diversity		Relative diversity		
	Total	Within samples	Within samples	Among samples within regions	Among regions
Four-region - all loci	10.757	10.717	0.996	0.002	0.002
Four-region - all loci, BC only	10.750	10.712	0.996	0.002	0.002
Four-region - six loci	5.277	5.261	0.997	0.002	0.001
Two-region - all loci	10.759	10.717	0.996	0.002	0.002
Two-region - all loci, BC only	10.75	10.712	0.996	0.002	0.002
Two-region – six loci	5.278	5.261	0.996	0.002	0.002

Table 5. Genetic variation in immature (≤50 mm SL), transition (51-69 mm SL), mature (70-99 mm SL) and fishery (>99 mm SL) northern abalone from five collection sites in British Columbia. The sample size (N), the mean number of alleles observed over all loci (A), the expected (H_e) and observed (H_o) heterozygosity levels estimated over all loci and the inbreeding coefficient (f_{is}) estimated from six loci at which genotypes were closest to HWE distributions are given for each size group.

Size	N	A	H_e	H_o	6 locus f_{is}
Louscoone					
immature	20	15.1	0.89	0.66	0.06
transition	66	24.8	0.89	0.69	0.08
mature	52	21.8	0.89	0.71	0.02
Denman					
mature	36	17.7	0.88	0.71	0.01
fishery	36	18.5	0.88	0.76	-0.02
Stryker					
transition	18	13.8	0.88	0.69	0.06
mature	70	22.6	0.88	0.70	0.04
West coast VI					
mature	46	20.0	0.89	0.64	0.19
fishery	70	24.4	0.89	0.72	0.07
Hankin Point					
mature	49	21.8	0.89	0.73	0.04
fishery	80	24.7	0.89	0.74	0.06

Figure 1. Sample locations of *Haliotis kamtschatkana* in British Columbia and Alaska.

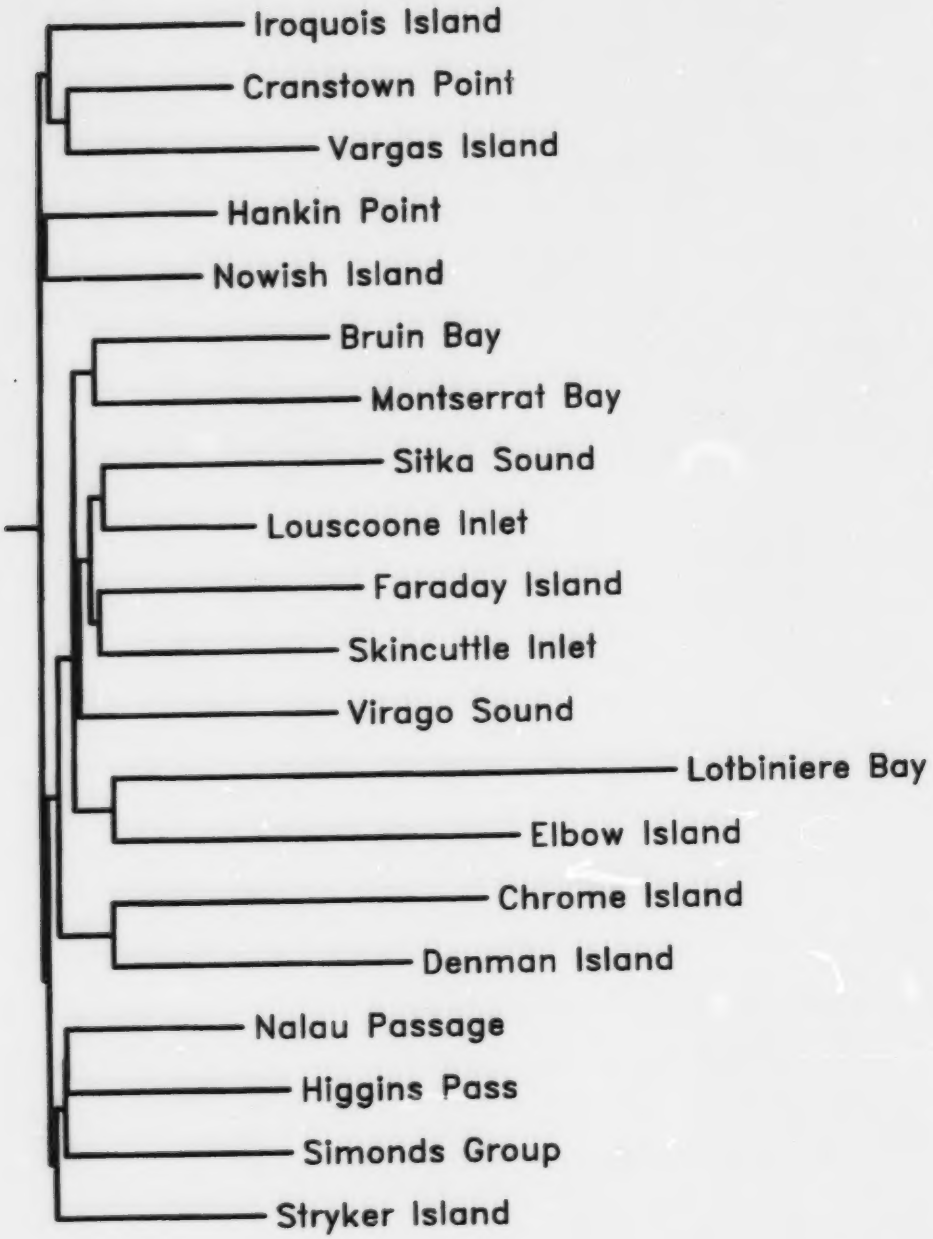

Figure 2. Neighbor joining dendrogram of *Haliotis kamtschatkana* samples based on Cavalli-Sforza and Edwards (1967) chord distance calculated from all 12 microsatellite loci.

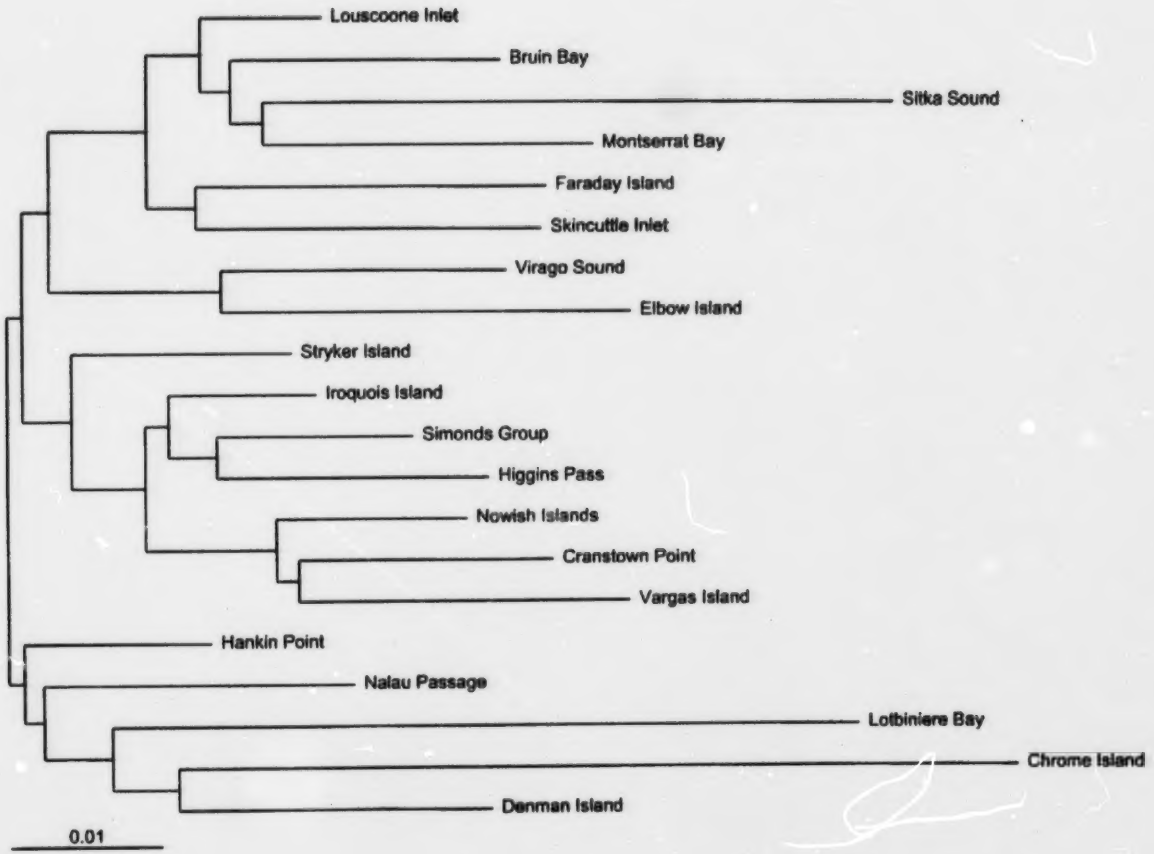

Figure 3. Neighbor joining dendrogram of *Haliotis kamtschatkana* samples based on Nei's (1972) genetic distance calculated from the six microsatellite loci for which genotypic frequencies were closest to HWE expectations.

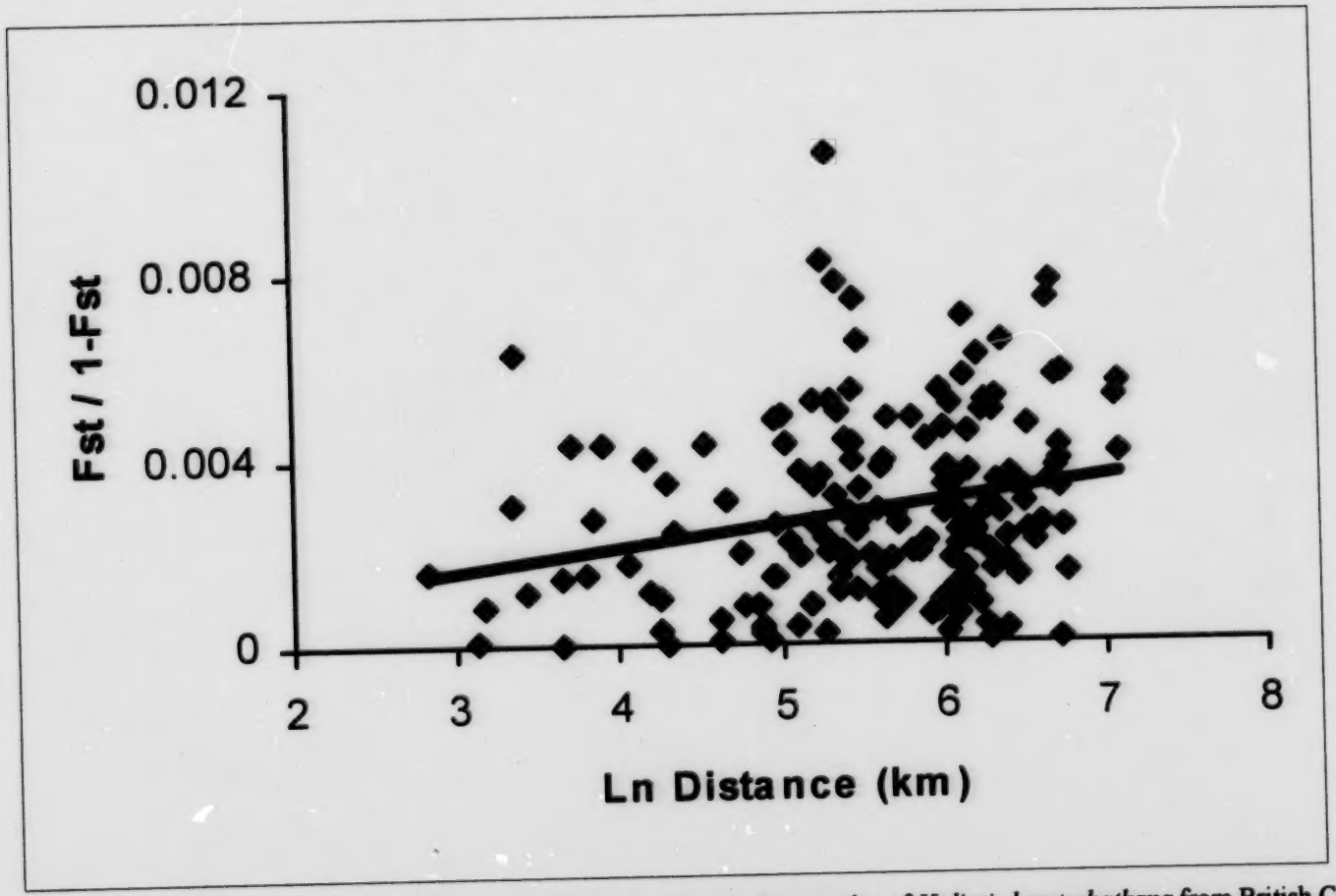

Figure 4. Relationship between $F_{ST}/(1-F_{ST})$ and geographic distance for samples of *Haliotis kamtschatkana* from British Columbia and southeast Alaska.